SARS-CoV-2 Variants in Rhode Island

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ABSTRACT

COVID-19 is a worldwide public health emergency caused by SARS-CoV-2. Genomic surveillance of SARS-CoV-2 emerging variants is important for pandemic monitoring and informing public health responses. Through an interstate academic-public health partnership, we established Rhode Island's capacity to sequence SARS-CoV-2 genomes and created a systematic surveillance program to monitor the prevalence of SARS-CoV-2 variants in the state. We describe circulating SARS-CoV-2 lineages in Rhode Island; provide a timeline for the emerging and expanding contribution of variants of concern (VOC) and variants of interest (VOI), from their first introduction to their eventual predominance over other lineages; and outline the frequent identification of known adaptively beneficial spike protein mutations that appear to have independently arisen in non-VOC/non-VOI lineages. Overall, the described Rhode Island-centric genomic surveillance initiative provides a valuable perspective on SARS-CoV-2 in the state and contributes data of interest for future epidemiological studies and state-to-state comparisons.

KEYWORDS: COVID-19, SARS-CoV-2, variants, public health, genomic sequencing, viral mutations

BACKGROUND

The 2019 coronavirus disease (COVID-19) pandemic has resulted in 180,569,875 infections and 3,912,211 deaths worldwide as of June 26, 2021.[1] Since publication of the first genomic sequence for the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2),[2] we have witnessed the development of viral mutations throughout the SARS-CoV-2 genome, with gradual predominance of those with major selective advantages.[3,4] These viruses with mutations, termed variants, though feared in the context of this and other pandemics, are expected as a natural part of virus life cycles.[5] Viruses, including SARS-CoV-2, mutate routinely as they evolve due to error-prone replication processes, during which mutations accumulate.[6] What matters most is whether these mutations have epidemiologic and clinical implications.

Driven by epidemiological necessity, genomic surveillance efforts increased towards the end of 2020 and it became increasingly clear that multiple SARS-CoV-2 variants with enhanced fitness were emerging independently in different parts of the world, including the United Kingdom,[7] South Africa,[8] Brazil,[9] California,[10] New York,[11] and most recently India.[12] Within a short time, the United States Centers for Disease Control and Prevention (CDC) defined, in an evolving process, viral variants of interest (VOI), with mutations of likely clinical and public health significance based on available data; variants of concern (VOC), with mutations of known significance; and variants of high consequence, with mutations of high significance, against which prior prevention and medical efforts fail.[13] Though existing nomenclature of SARS-CoV-2 variants remains a challenge,[14] the World Health Organization (WHO) recently proposed a simplification,[15] and, as of this writing, CDC-defined VOI include lineages B.1.427 and B.1.429 (both WHO Epsilon), first identified in California; B.1.525 (Eta) and B.1.526 (Iota), first identified in New York; B.1.617.1 (Kappa) and B.1.617.3 in India; and P.2 (Zeta) in Brazil; and VOC include lineages B.1.1.7 (Alpha), first identified in the United Kingdom; B.1.351 (Beta) in South Africa; B.1.617.2 (Delta) in India; and P.1 (Gamma) in Brazil. There are no currently defined variants of high consequence.[13]

VOC and VOI are elevated to these designations from conventional viral variants due to their mutations with clinical and public health impact. Though these mutations occur throughout the ~30,000 nucleotide span of the SARS-CoV-2 genome, the most strategically located ones are in or close to the receptor binding domain (RBD) of the viral spike protein. This specific location mediates the viral binding to the angiotensin-converting enzyme 2 (ACE2) human cellular receptor, and the resulting membrane fusion and viral replication cycle, making it also a prime target of innate and adaptive antiviral responses.[16]

As of June 25, 2021, Rhode Island (RI) has had a total of 152,514 positive COVID-19 cases, and 2,728 associated deaths.[17] In April 2020, the Kantor laboratory at the Providence-Boston Center for AIDS Research set up the capacity for SARS-CoV-2 whole genome sequencing to examine variant and mutation evolution in RI.[18] This initial, academic interest has been since formalized and significantly enhanced by the RI State Health Laboratory (RISHL), and regional and

national collaborators, aided by the large genomic surveillance investment and analytic tools development in the United States and globally.[19-22] In this manuscript we present the evolving status of the large-scale surveillance of SARS-CoV-2 variants in RI, raise awareness to their existence, and discuss their potential implications for public health responses, as we continue to fight this global pandemic.

METHODS

Collection of SARS-CoV-2 samples

The RISHL established a system for the collection of residual specimens from clinical laboratories involved in SARS-CoV-2 diagnostic testing for RI residents. The system was designed to provide specimens from infected individuals who were hospitalized, reside or work at long-term care facilities, correctional facilities staff and inmates, K–12 school students and staff, colleges and universities, and the general population seeking SARS-CoV-2 testing. Samples were submitted to the RISHL, de-identified and sequenced by collaborating laboratories, including the CDC, Broad Institute and Kantor Laboratory. The RISHL also established an independent SARS-CoV-2 sequencing capacity. In a parallel effort, CDC contracted several commercial laboratories to sequence SARS-CoV-2 from diagnostic specimens. Sequences generated in these laboratories are submitted to public databases, including the Global Initiative on Sharing All Influenza Data (GISAID).[20] The work presented here includes sequences originating from RI residents that were aggregated from GISAID.

SARS-CoV-2 sequencing and sequence analysis

Sequencing methods varied by individual protocols of laboratories wherein samples were processed. For illustration, in the Kantor laboratory and RISHL, for specimens with low (<30) cycle thresholds (Ct), RNA was extracted, reverse transcribed and amplified, and the entire SARS-CoV-2 genome was sequenced by next generation sequencing (NGS) using the Illumina MiSeq platform. Genomic analyses of statewide SARS-CoV-2 sequences were conducted at the Kantor laboratory, with available tools and custom python scripts.[21-23] Scripts used for analyses pipelines are available under an open-source license from https://github.com/kantorlab/covid-pipeline.

Analysis of SARS-CoV-2 mutations

To investigate development of viral mutations outside known VOC/VOI, we first examined occurrence of SARS-CoV-2 spike protein mutations that have been associated with a VOC or VOI by CDC definitions,[13] but that occurred at least once in variants *not* classified as VOC or VOI. We examined the five most frequent mutations and aggregated the remaining as 'other'. We also specifically examined occurrence of VOC/VOI mutations between amino acid positions 417–501 in the spike RBD, which have been more commonly associated with poor clinical outcomes like increased transmission and disease severity, and immune escape.

Phylogenetic analysis

To provide a snapshot of the phylogenetic spectrum of available RI SARS-CoV-2 sequences from the start of the COVID-19 pandemic, we created a maximum likelihood tree with RAxML,[24] that includes the earliest (i) RI non-VOC/non-VOI sequence per month since the start of the epidemic; (ii) VOC sequence per month since its detection in RI; (iii) VOI sequence per month since its detection in RI; the earliest and latest available non-VOC/non-VOI sequences from each New England state (Maine, New Hampshire, Vermont, Massachusetts, Connecticut) and New York; one reference sequence for each of the VOI/VOC; and the original SARS-CoV-2 sequence from Wuhan (used as a root). The number of sequences included was limited to allow a reasonable tree resolution.

RESULTS

The first COVID-19 case was detected at the RISHL on 2/29/20 and sequenced by the CDC. A selection of succeeding isolates provided by the RISHL to the Kantor laboratory and the CDC were further sequenced through December of 2020 (n=99). December of 2020 was punctuated by a heightened concern over the B.1.1.7 variant that was emerging in the USA and it was recognized that a (albeit nonspecific) marker for B.1.1.7 was that it displayed a "S-gene target failure" (SGTF) profile by some real-time PCR assays.[25] During that time, the RISHL began to see an alarming increase in the number of positive specimens that were SGTF and rapidly scaled up sequencing efforts in order to ascertain whether B.1.1.7 was circulating in RI. It was not B.1.1.7 yet, as most of the November–December 2020 SGTF lineage was actually the B.1.375 variant.[25] It was not until 1/19/21 that the first B.1.17 variant was detected in RI. The expansion of sequencing efforts succeeded thanks to regional collaborations with the Massachusetts Department of Health and the Sabeti Laboratory at the Broad Institute, Cambridge, MA. Indeed, from December 2020 through June 2021 the number of SARS-CoV-2 isolates successfully sequenced from RI residents by the collaborative has substantially increased by almost 40-fold and, as of 6/24/21, 3,963 SARS-CoV-2 RI sequences were available (**Figure 1, see Appendix**).

Given RI's proximity to major US population centers, the observed wide diversity of SARS-CoV-2 lineages was not surprising. Indeed, of the total 3,963 RI sequences available as of this writing, 1,489 (38%) are VOC, 1,177 (30%) are VOI, and 1,297 (33%) are considered non-VOC/non-VOI (**Table 1**).

Phylogenetic analyses of the RI SARS-COV-2 spectrum throughout the COVID-19 pandemic demonstrated expected clustering by VOC/VOI, dispersion of RI sequences among

Table 1. SARS-CoV-2 Variants of Concern / Interest in RI as of June 24, 2021.

Variant of Concern	Region Variant Originally Identified	Number of Total Cases	Range of Sampling Dates
B.1.1.7 (Alpha)	UK	1248	Jan 19 to Jun 05, 2021
B.1.351 (Beta)	South Africa	8	Mar 16 to May 25, 2021
B.1.617.2 (Delta)	India	9	Apr 20 to May 25, 2021
P.1 (Gamma)	Brazil	224	Mar 03 to Jun 03, 2021
Variant of Interest	**Region Variant Originally Identified**	**Number of Total Cases**	**Range of Sampling Dates**
B.1.427 (Epsilon)	California, USA	41	Jan 27 to Apr 15, 2021
B.1.429 (Epsilon)	California, USA	94	Jan 06 to Apr 29, 2021
B.1.525 (Eta)	New York, USA	51	Feb 03 to Apr 27, 2021
B.1.526 (Iota)	New York, USA	991	Jan 07 to Jun 03, 2021
B.1.617.1 (Kappa)	India	0	—
B.1.617.3	India	0	—
P.2 (Zeta)	Brazil	0	-—

regional sequences, and evolution of mutations over time. This latter point is indicated by early sequences in the pandemic being closer to the root of the phylogenetic tree, and more recent sequences being more distal descendants of the original Wuhan strain (**Figure 2, see Appendix**).

Multiple and diverse non-VOC/non-VOI lineages have been circulating in RI throughout the pandemic, with changing proportions over time (**Figures 3A, 3B, see Appendix**). Only few lineages such as B.1.375 and B.1.2 made up substantial numbers of non-VOC/non-VOI sequences at any one time, with considerable subsequent declines of B.1.375 and B.1.2. Before January 2021, none of the then available sequences (n=197) would be considered VOC/VOI.

Between January 2021 and May 2021, a steadily increasing proportion of sequenced RI samples were VOC/VOI; from 12/205 (6%) in January to 684/771 (89%) in May 2021 (**Figure 3A**). Indeed, on a weekly basis, from January 2021, VOC/VOI proportions have steadily increased, effectively supplanting non-VOC/non-VOI lineages (**Figure 3C, see Appendix**). For example, of the 109 RI samples sequenced in the week of 5/23/21-5/29/21, 72 (66%) are VOC and 23 (21%) are VOI. **Table 1** lists cases identified as VOCs and VOIs at the time of this writing. Current data are maintained by the RI Department of Health and are available at https://ri-department-of-health-covid-19-variant-data-rihealth.hub.arcgis.com/. Overall, all four VOCs (B.1.1.7, B.1.351, B.1.617.2 and P.1) and four (B.1.427, B.1.429, B.1.525 and B.1.526) of the seven defined VOIs were detected in RI (**Table 1; Figure 3C**). The first VOC detected in RI was B.1.429 in January 2021. The most frequently occurring VOC is B.1.1.7, first sequenced 1/19/21, then growing rapidly to 31% of sequences by June 5, 2021. Other common lineages include VOC P.1 and VOI B.1.526. As of this writing, B.1.1.7, P.1, and B.1.526 have each remained at an overall consistent proportion of all identified cases from the weeks of April 4 to May 29, 2021: 48% to 48%, 4% to 15%, and 30% to 21%, respectively (**Figure 3C**).

Multiple spike protein mutations associated with certain VOC and/or VOI also occurred in RI sequences from non-VOC/non-VOI lineages. Out of 53 spike mutations/deletions associated with any VOC/VOI per CDC,[13] 41 occurred in at least one RI sequence that was not the VOC/VOI with which that mutation is most commonly associated (**Figure 4, see Appendix**). The five most prevalent mutations included L5F (associated with B.1.526; n=828), T95I (B.1.526, B.1.617.1; n=788), D253G (B.1.526; n=774), S477N (B.1.526; n=742), and V1176F (P.2; n=243).

Of nine mutations at seven positions within the SARS-CoV-2 RBD (positions 417-501) associated with VOC/VOI, seven (including S477N, as above) occurred outside of their VOC/VOI associated lineages, including K417T (associated with P.1; n=61), L452R (B.1.427, B.1.429, B.1.526.1, B.1.617, B.1.617.1, B.1.617.2, B.1.617.3; n=46), T478K (B.1.617.2; n=27), E484K (B.1.1.7, B.1.351, B.1.525, B.1.526, P.1, P.2; n=191), S494P (B.1.1.7; n=81), and N501Y (B.1.1.7, B.1.351, P.1; n=113) (**Figure 5, see Appendix**). The proportion of mutations in spike protein sequences overall and within the RBD shifted over time from an initial predominance of other spike mutations like the H69-V70 deletion, to an increasing input of L5F, T95I, D253G, S477N, and V1176F detected mutations (**Figures 4, 5**).

DISCUSSION

We present current data on SARS-CoV-2 genomic surveillance in RI, demonstrating an exponential increase in statewide sequencing capacity, heterogenous lineage dynamics, overtake of the sequence landscape by VOC and VOI during March/April of 2021, and continued evolution of mutations of significance in non-VOC/non-VOI lineages. Accumulating and routinely updated data on SARS-CoV-2 VOC/VOI in RI can be seen in a website maintained by the authors and the RI Department of Health.[23] Though these observations may not be unique to RI, a statewide comprehensive approach is, and it continues to enable monitoring, linking to epidemiology and investments in genomic surveillance within the state. Such efforts will ensure increased awareness, optimal understanding, and efficient public health responses to this evolving pandemic as we continue to implement vaccination and prepare for pre-COVID-19 era normalcy.

Of 3,963 SARS-CoV-2 RI sequences by the end of June 2021, most common lineages increasingly belong to VOC B.1.1.7 and to a lesser extent VOC P.1 and VOI B.1.526, as in the other northeastern USA states.[26] Most other VOC/VOI

(except P.2, B.1.617.1 and B.1.617.3) have also been detected in RI, some in small numbers. Global challenges associated with these VOC/VOI continue to be encountered, including increased transmissibility,[27] more severe disease outcomes,[28] re-infection,[29] and vaccine and treatment evasions.[30,31] At this point in time, the impact of knowing variant designation on an individual clinical level is limited, primarily due to delay in obtaining sequences. However, on a population level this information continues to be important, and it can inform treatment, prevention, and public health.[32] Interpretation of specific variant-outcome associations data should be done with caution, considering their continuous evolution.

VOC and VOI can be transmitted through travel, migration, and immigration. However, as we show, mutations of significance that are in these VOC/VOI, including those at strategic viral locations that may have negative functional impact (e.g. increased transmissibility via improved affinity of the viral RBD to the human ACE2 receptor, or immune evasion by decreased human antibody binding to the viral RBD) can also develop *de novo*, most likely by selective pressure from the immune system, treatments and vaccines.[4] This continues to be concerning, and as currently seen in India, the emergence of new variants can be fueled by high population density and insufficient vaccinations, allowing abundant viral replication and mutation evolution and fixation.[33]

A partnership of multiple academic labs, including the Kantor and Sabeti Laboratories, and the RI Department of Health State Health Laboratories is at the basis of this successful establishment of SARS-CoV-2 genomic surveillance in RI. This existing RI collaboration was in place prior to the COVID-19 pandemic, and mostly focused on HIV genomics and investigation of methods to minimize HIV transmission.[34-37] Leveraging these collaborations, we have established SARS-CoV-2 data generation, quality control, bioinformatics pipelines, interpretation and reporting systems that inform public health and allow integration with epidemiological and geographic data for the benefit of RI residents. Such intra-state and regional processes augment nationwide and global efforts to monitor and respond to the COVID-19 pandemic as it develops.

SARS-CoV-2 genomic surveillance is challenging, and limitations exist. Accurate and representative population sampling is difficult, particularly considering that successful sequencing is only feasible for certain samples, i.e., those with relatively low Ct's, representing high viral levels. Reporting of sequencing data to public health is not yet standardized and requires substantial efforts to combine with relevant demographic data. Additionally, rapid development of pandemic-related events and SARS-CoV-2 genome sequencing efforts have made it challenging to agree on nomenclature, lineage delineation, genomic quality control measures, and relevant mutation lists, to name a few.

In conclusion, SARS-CoV-2 VOC and VOI have become dominant in RI, as well as in the surrounding New England states and the rest of the country, which deserves awareness from the general RI population and health providers. The continued decrease in new cases in RI despite the proportional predominance of VOC/VOI is encouraging and can most likely be attributed to optimized public health measures and high (~75% of those >18 years) vaccination rate in the state. SARS-CoV-2 is still circulating, particularly globally, and we must continue to be aware, understand and monitor evolving SARS-CoV-2 variants, especially those that become of concern or interest. Conventional mitigation measures that have been used throughout this pandemic, including masking, social distancing, avoiding large gatherings, decreasing travel, and increasing testing, quarantining and contact tracing, remain available in the event of variant-induced surge of cases. Importantly, ensuring global availability of vaccinations, and continuing focus on high vaccination rates in the US, will protect us all and allow us to get back to our pre-pandemic lives.

Appendix

References

1. Johns Hopkins COVID-19 Dashboard. Accessed 6/26/21, https://coronavirus.jhu.edu/
2. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. Mar 2020;579(7798):270-273. doi:10.1038/s41586-020-2012-7
3. Korber B, Fischer WM, Gnanakaran S, et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. *Cell*. Aug 20 2020;182(4):812-827 e19. doi:10.1016/j.cell.2020.06.043
4. Harvey WT, Carabelli AM, Jackson B, et al. SARS-CoV-2 variants, spike mutations and immune escape. *Nat Rev Microbiol*. Jul 2021;19(7):409-424. doi:10.1038/s41579-021-00573-0
5. Grubaugh ND, Petrone ME, Holmes EC. We shouldn't worry when a virus mutates during disease outbreaks. *Nat Microbiol*. Apr 2020;5(4):529-530. doi:10.1038/s41564-020-0690-4
6. Callaway E. The coronavirus is mutating - does it matter? *Nature*. Sep 2020;585(7824):174-177. doi:10.1038/d41586-020-02544-6
7. Wise J. Covid-19: New coronavirus variant is identified in UK. *BMJ*. Dec 16 2020;371:m4857. doi:10.1136/bmj.m4857
8. Tegally H, Wilkinson E, Giovanetti M, et al. Detection of a SARS-CoV-2 variant of concern in South Africa. *Nature*. Apr 2021;592(7854):438-443. doi:10.1038/s41586-021-03402-9
9. Faria NR, Mellan TA, Whittaker C, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science*. May 21 2021;372(6544):815-821. doi:10.1126/science.abh2644
10. Zhang W, Davis BD, Chen SS, Sincuir Martinez JM, Plummer JT, Vail E. Emergence of a Novel SARS-CoV-2 Variant in Southern California. *JAMA*. Apr 6 2021;325(13):1324-1326. doi:10.1001/jama.2021.1612
11. Annavajhala MK, Mohri H, Zucker JE, et al. A Novel SARS-CoV-2 Variant of Concern, B.1.526, Identified in New York. *medRxiv*. Feb 25 2021;doi:10.1101/2021.02.23.21252259
12. Yadav PD, Sapkal GN, Abraham P, et al. Neutralization of variant under investigation B.1.617 with sera of BBV152 vaccinees. *Clin Infect Dis*. May 7 2021;doi:10.1093/cid/ciab411

13. CDC. SARS-CoV-2 Variant Classifications and Definitions. Accessed 5/12/21, https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html
14. Callaway E. 'A bloody mess': Confusion reigns over naming of new COVID variants. *Nature*. Jan 2021;589(7842):339. doi:10.1038/d41586-021-00097-w
15. WHO. Tracking SARS-CoV-2 variants. Accessed 6/28/21, https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/
16. V'Kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. *Nat Rev Microbiol*. Mar 2021;19(3):155-170. doi:10.1038/s41579-020-00468-6
17. RIDOH COVID-19. Accessed 6/28/21, https://ri-department-of-health-covid-19-data-rihealth.hub.arcgis.com/
18. Brown University COVID-19 seed awards. Accessed 6/28/21, https://www.brown.edu/research/conducting-research-brown/finding-funding/internal-funding-opportunities/brown-covid-19-research-seed-fund-awardees
19. White House COVID-19 genomic surveillance funding. Accessed 6/28/21, https://www.whitehouse.gov/briefing-room/statements-releases/2021/04/16/fact-sheet-biden-administration-announces-1-7-billion-investment-to-fight-covid-19-variants/
20. GISAID. Accessed 6/28/21, https://www.gisaid.org/
21. Pangolin. Accessed 6/28/21, https://cov-lineages.org/
22. Nextstrain. Accessed 6/28/21, https://nextstrain.org/
23. RIDOH COVID-19 variants. Accessed 6/28/21, https://ri-department-of-health-covid-19-variant-data-rihealth.hub.arcgis.com/
24. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. Feb 7 2014;doi:btu033[pii]10.1093/bioinformatics/btu033
25. Vogels CBF, Breban MI, Ott IM, et al. Multiplex qPCR discriminates variants of concern to enhance global surveillance of SARS-CoV-2. *PLoS Biol*. May 2021;19(5):e3001236. doi:10.1371/journal.pbio.3001236
26. CDC COVID-19 data tracker. Accessed 6/28/21, https://covid.cdc.gov/covid-data-tracker/#variant-proportions
27. Davies NG, Abbott S, Barnard RC, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. *Science*. Apr 9 2021;372(6538)doi:10.1126/science.abg3055
28. Davies NG, Jarvis CI, Group CC-W, et al. Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. *Nature*. May 2021;593(7858):270-274. doi:10.1038/s41586-021-03426-1
29. Nonaka CKV, Franco MM, Graf T, et al. Genomic Evidence of SARS-CoV-2 Reinfection Involving E484K Spike Mutation, Brazil. *Emerg Infect Dis*. May 2021;27(5):1522-1524. doi:10.3201/eid2705.210191
30. Garcia-Beltran WF, Lam EC, St Denis K, et al. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell*. Apr 29 2021;184(9):2523. doi:10.1016/j.cell.2021.04.006
31. Zhou D, Dejnirattisai W, Supasa P, et al. Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. *Cell*. Apr 29 2021;184(9):2348-2361 e6. doi:10.1016/j.cell.2021.02.037
32. Chen RE, Zhang X, Case JB, et al. Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies. *Nature medicine*. Apr 2021;27(4):717-726. doi:10.1038/s41591-021-01294-w
33. Mallapaty S. India's massive COVID surge puzzles scientists. *Nature*. Apr 2021;592(7856):667-668. doi:10.1038/d41586-021-01059-y
34. Alexander NE, Chan PA, Rogo TO, et al. Interrupting transmission of HIV and other sexually transmitted infections in Rhode Island. *Med Health R I*. Aug 2012;95(8):241-4.
35. Chan PA, Kazi S, Rana A, et al. Short communication: new HIV infections at Southern New England academic institutions: implications for prevention. *AIDS Res Hum Retroviruses*. Jan 2013;29(1):25-9. doi:10.1089/AID.2012.0130
36. Novitsky V, Steingrimsson JA, Howison M, et al. Empirical comparison of analytical approaches for identifying molecular HIV-1 clusters. *Sci Rep*. Oct 29 2020;10(1):18547. doi:10.1038/s41598-020-75560-1
37. Novitsky V, Steingrimsson J, Howison M, et al. Longitudinal typing of molecular HIV clusters in a statewide epidemic. *AIDS*. May 24 2021;doi:10.1097/QAD.0000000000002953

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Disclaimer

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